# BsrDI

REF: EG25506S


Isoschizomers*: Bse3DI, BseMI

*Isoschizomers may have different methylation sensitivities.

37 EB 20 80 ★

## Storage Condition

-20℃

## Components

| Component | Amount |
|---|---|
| BsrDI (10 U/μl) | 25 μl |
| 10× Cut Buffer B | 1 ml |

## Description

BsrDI is derived from *Bacillus stearothermophilus* D70 and consists of two subunits of different sizes, belonging to a heterodimeric enzyme. BsrDI recognizes the specific sequence GCAATG/CATTGC. When cutting the top strand, it cuts at a position 2 nucleotides downstream of the recognition site, while for the bottom strand, it cuts immediately, after the recognition site, generating specific sticky ends. BsrDI belongs to the Type IIS restriction enzymes, which recognize asymmetric DNA sequences and cleave outside of their recognition sequence, making it suitable for Golden gate assembly.

## Recommended Reaction Conditions

1× Cut Buffer B;
Incubate at 37℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.

## Heat Inactivation

Incubation at 80℃ for 20 minutes.

## Definition of Activity Unit

One unit of activity refers to the amount of enzyme required to completely digest 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour.

## Quality Control Assays

**Function**

10 U of BsrDI can completely digest 1 μg of λDNA within 15 minutes at 37℃ .

**Prolonged Incubation / Star Activity Assay**

Under optimal reaction temperature, incubate 10 U BsrDI with 1 μg λDNA for 3 hours. No contamination from other nucleases or non-specific substrate degradation caused by star activity was detected. Longer incubation may result in star activity.

**Ligation and Recuting**

Under optimal reaction temperature, digest the substrate using 10 U BsrDI and recover the digested products. >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃ . Of these ligated fragments, >95% can be recut with BsrDI as determined by agarose gel electrophoresis.

## Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

EB Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the EcoBI methylase.

20 80 The enzyme can be heat inactivated at by incubation 80℃ for 20 minutes.

★ 3 hours incubation did not show star activity, but delayed enzyme digestion might show star activity.



Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

# Protocol

## 1. Protocol for DNA Digestion

① Combine the following components on ice in the following order:

| Reagents | Volume |
|---|---|
| $ddH_2O$ | up to 50 µl |
| 10× Cut Buffer B | 5 µl |
| DNA[a] | 1 µg |
| BsrDI (10 U/µl) | 1 µl |
| Total | 50 µl |

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

② Mix gently and spin down.
③ Incubate at 37℃ for 15 minutes~1 hour.
④ Optional: Inactivate the enzyme by heating at 80℃ for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

## 2. Notice

① The volume of enzyme added to the reaction mixture should not exceed 10% of the total volume to avoid star activity caused by excessive glycerol in the enzyme storage buffer.
② The additives (e.g., glycerol, salt) in the enzyme storage buffer are the same as the contaminants in the substrate solution (e.g., salt, EDTA, or ethanol, etc.). Therefore, the smaller the reaction volume, the stronger the digestion inhibition effect.
③ BsrDI can be used in CutOne® Buffer, but the star activity is significantly stronger than in reactions performed Cut Buffer B. If using CutOne® Buffer for the reaction, it is not recommended that the digestion time exceed 3 hours or the amount of BsrDI exceed 2 U/µg of DNA substrate.

# Number of Recognition Sites in DNA

| λDNA | ΦX174 | pBR322 | pUC57 | pUC18/19 | SV40 | M13mp18/19 | Adeno2 |
|---|---|---|---|---|---|---|---|
| 44 | 4 | 2 | 2 | 2 | 4 | 3 | 14 |

# Methylation Effects on Digestion

| Dam | Dcm | CpG | EcoKI | EcoBI |
|---|---|---|---|---|
| No effect | No effect | No effect | No effect | Some blocked |

# Activity in Different Buffers*

| | CutOne® Buffer | Thermo Scientific FastDigest Buffer | NEB rCutSmart™ Buffer | Takara QuickCut™ Buffer |
|---|---|---|---|---|
| Activity | 50% | 100% | 50% | 100% |

*The activity data come from the functional test described above.



Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com